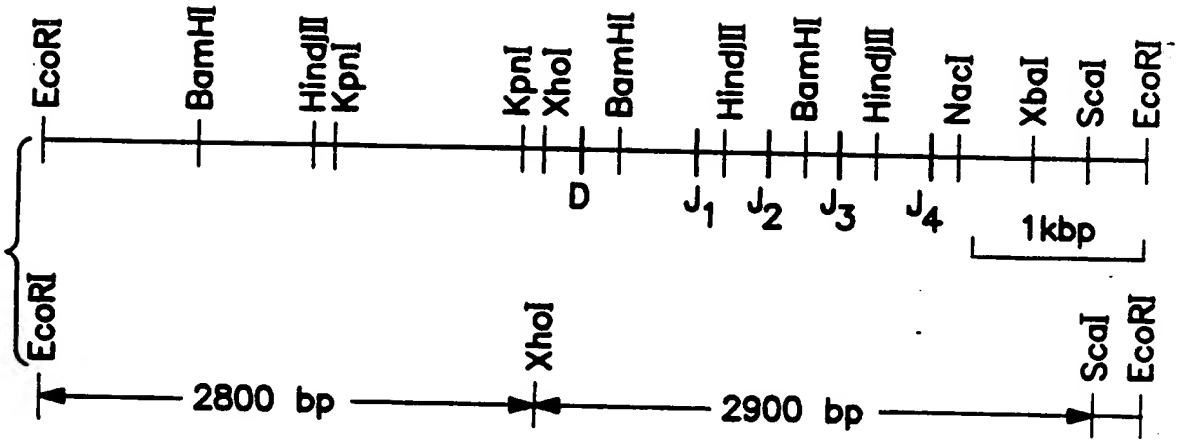

EcoRI
BamHI
HindIII
KpnI
KpnI
XhoI
BamHI
HindIII
BamHI
HindIII
NaeI
XbaI
ScaI
EcoRI
D
J1
J2
J3
J4
1kbp
EcoRI
XhoI
ScaI
EcoRI
2800 bp
2900 bp

FIG. IA

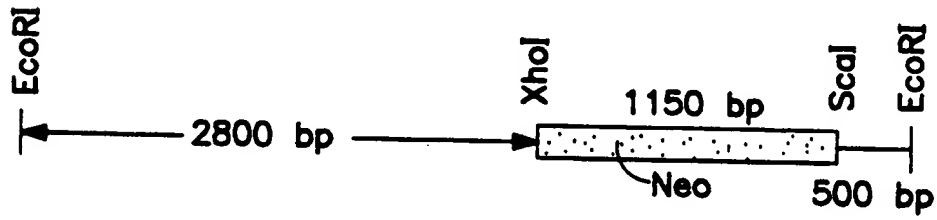

EcoRI
XhoI
1150 bp
ScaI
EcoRI
2800 bp
Neo
500 bp

FIG. IB

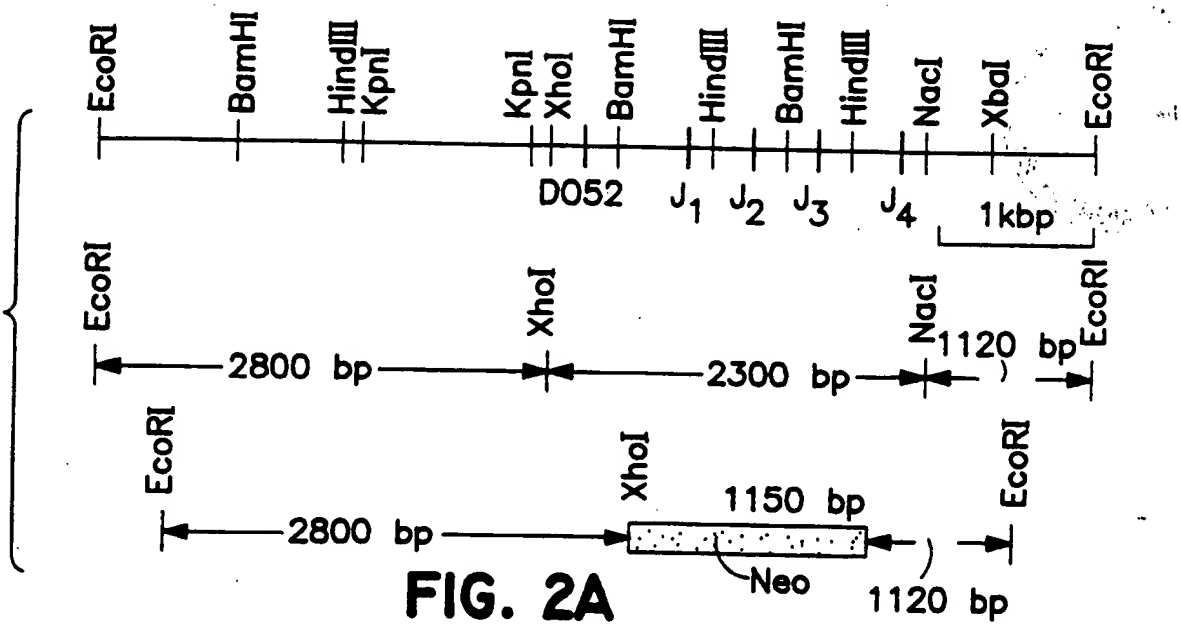



FIG. 2A

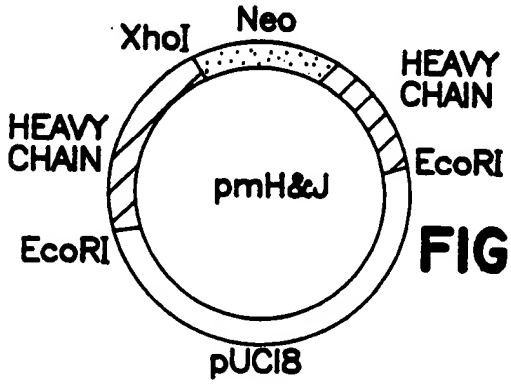



FIG. 2B

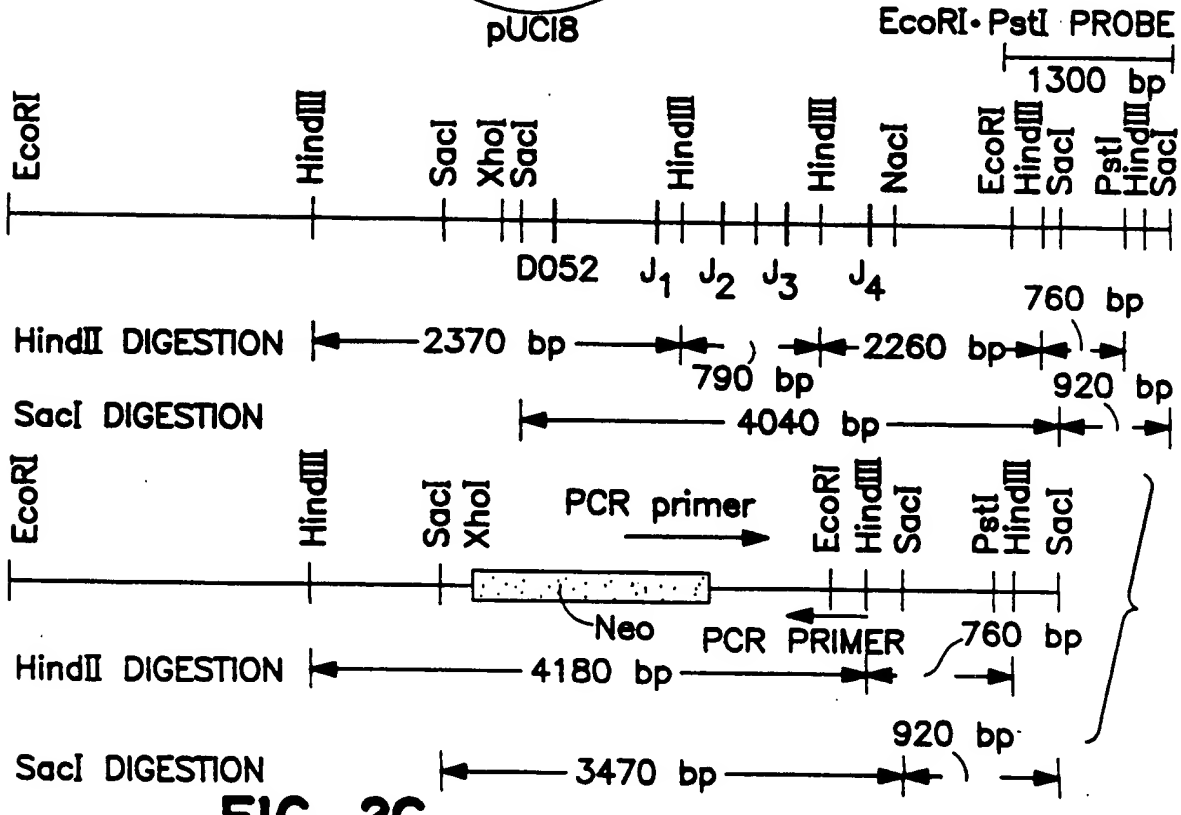



FIG. 2C

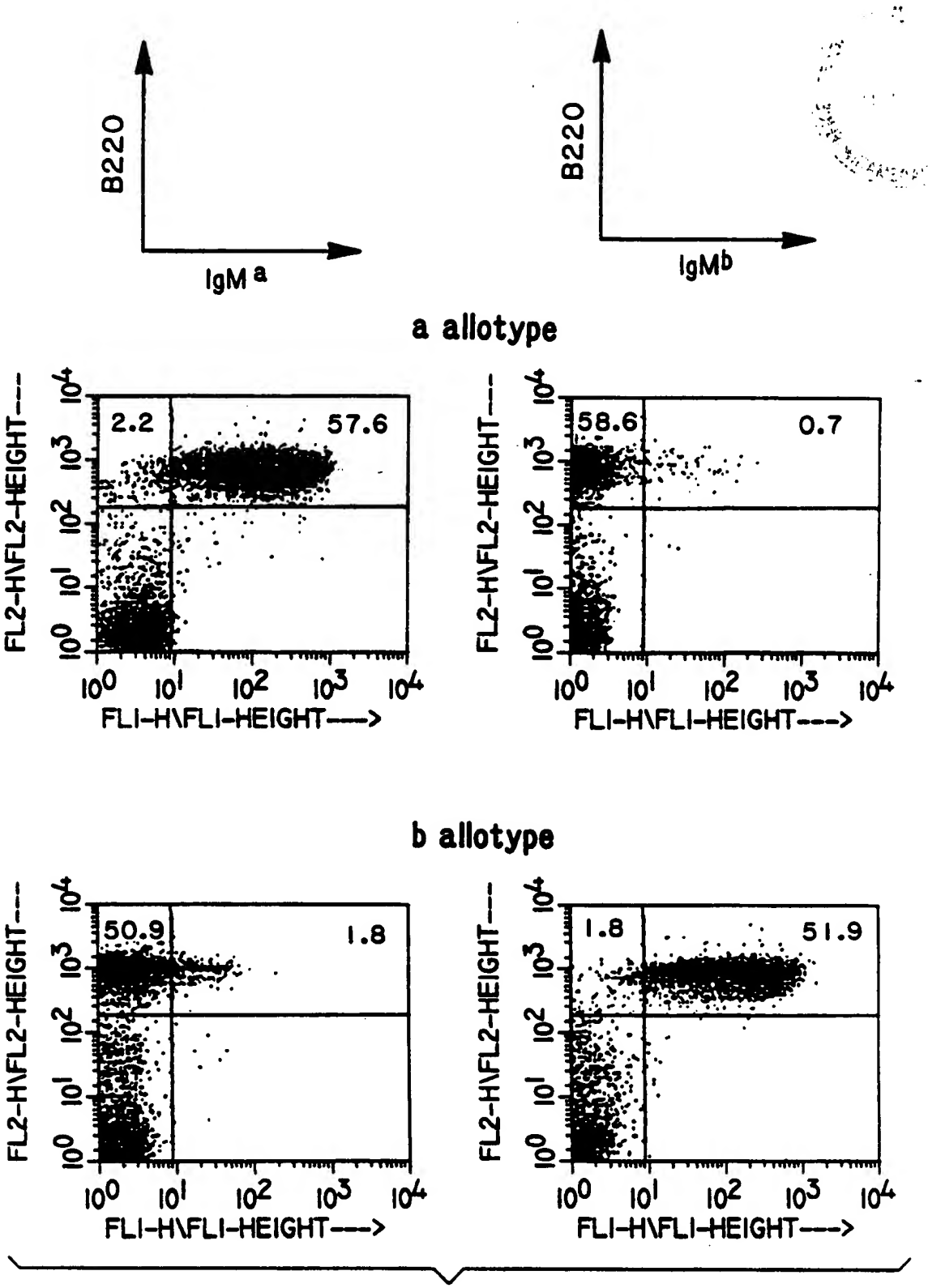

B220
IgM a
B220
IgMb
a allotype
FL2-H\FL2-HEIGHT---
FLI-H\FLI-HEIGHT--->
2.2
57.6
58.6
0.7
b allotype
50.9
1.8
1.8
51.9

FIG. 3-1

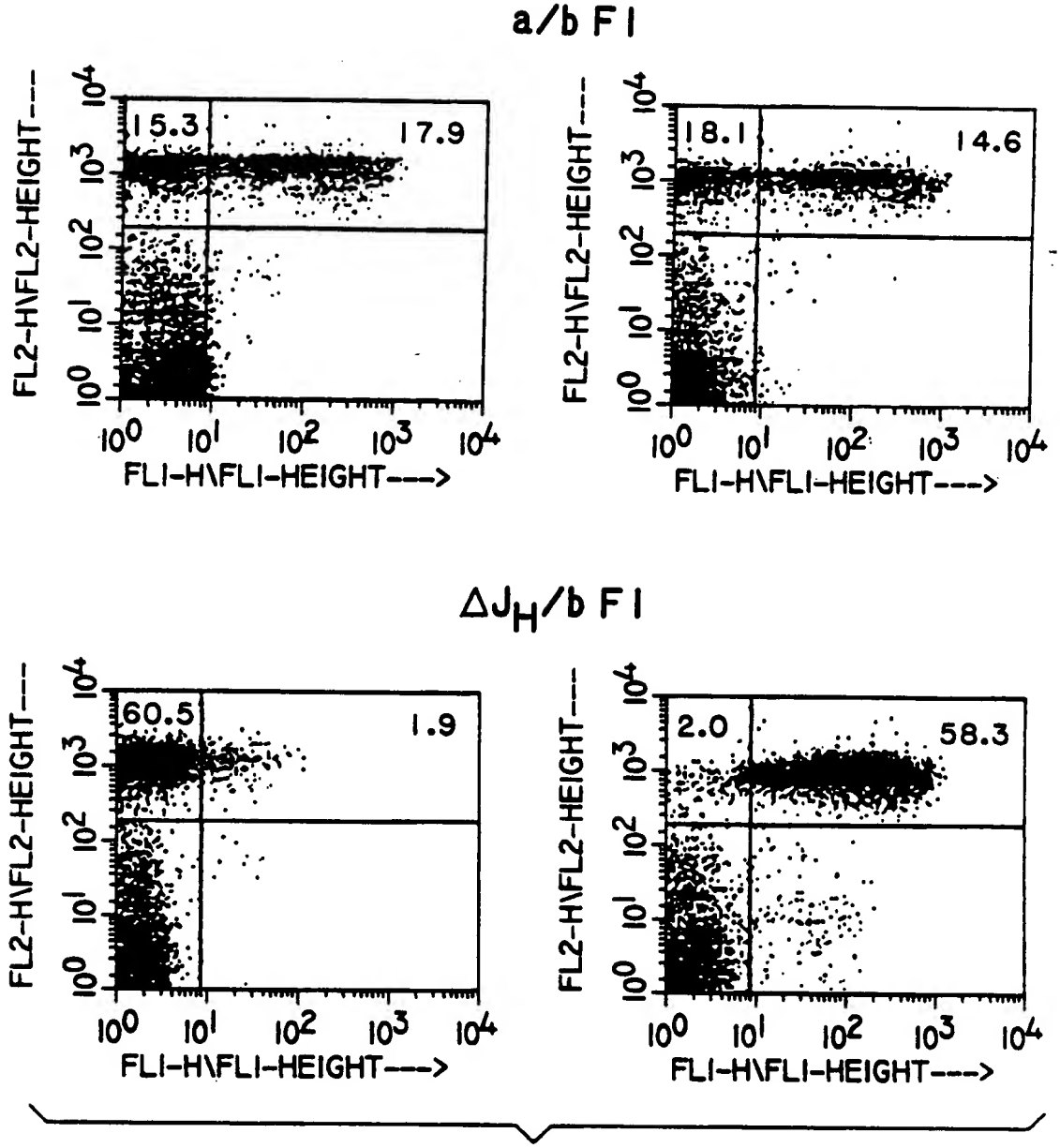

a/b F1
FL2-H\FL2-HEIGHT---
FLI-H\FLI-HEIGHT--->
15.3
17.9
18.1
14.6
ΔJH/b F1
60.5
1.9
2.0
58.3

FIG. 3-2

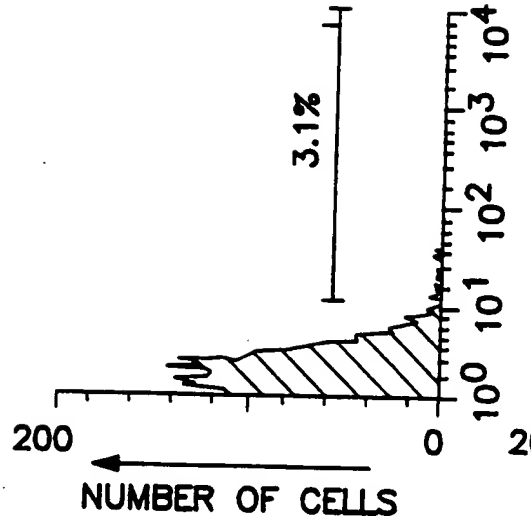



FIG. 4C

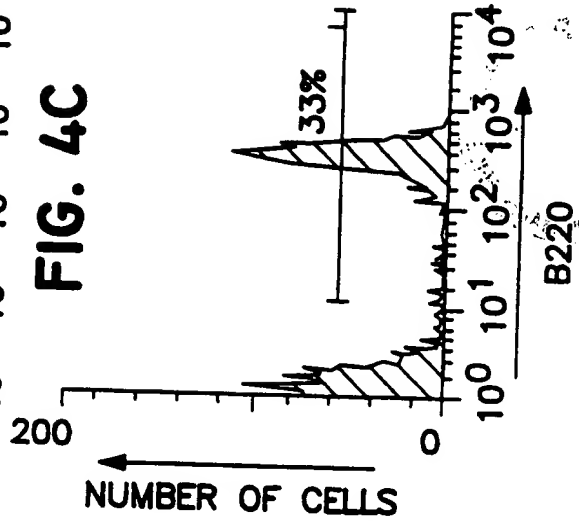



FIG. 4F

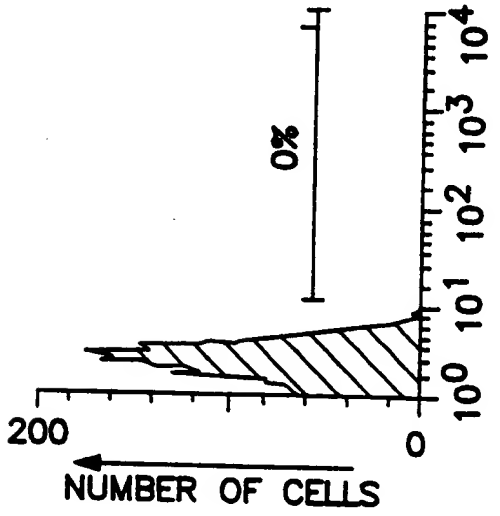



FIG. 4B

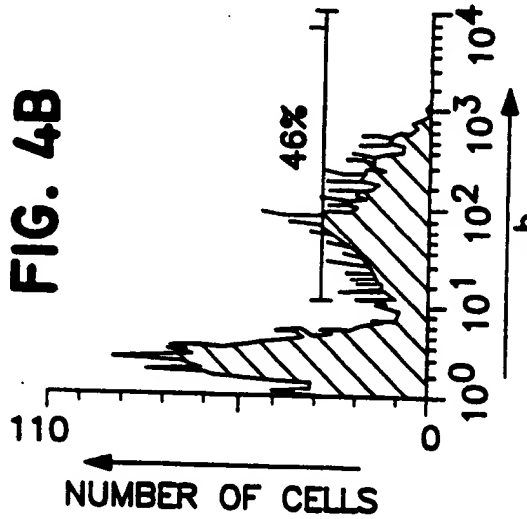



FIG. 4E

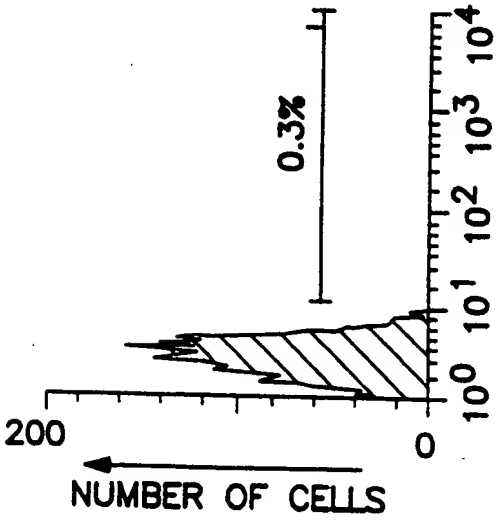



FIG. 4A

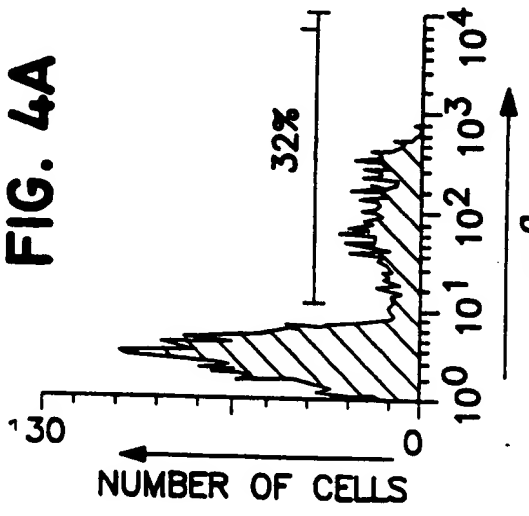



FIG. 4D

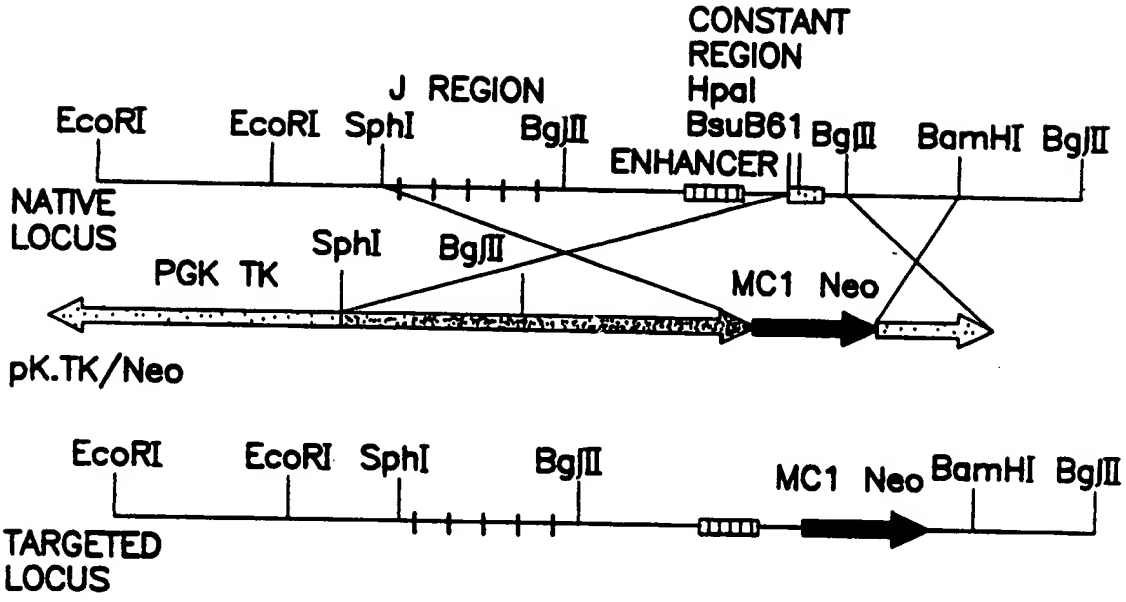

CONSTANT
REGION
HpaI
BsuB61
J REGION
EcoRI
EcoRI
SphI
BglII
ENHANCER
BglII
BamHI
BglII
NATIVE
LOCUS
SphI
BglII
PGK
TK
MC1
Neo
pK.TK/Neo
EcoRI
EcoRI
SphI
BglII
MC1
Neo
BamHI
BglII
TARGETED
LOCUS

FIG. 5

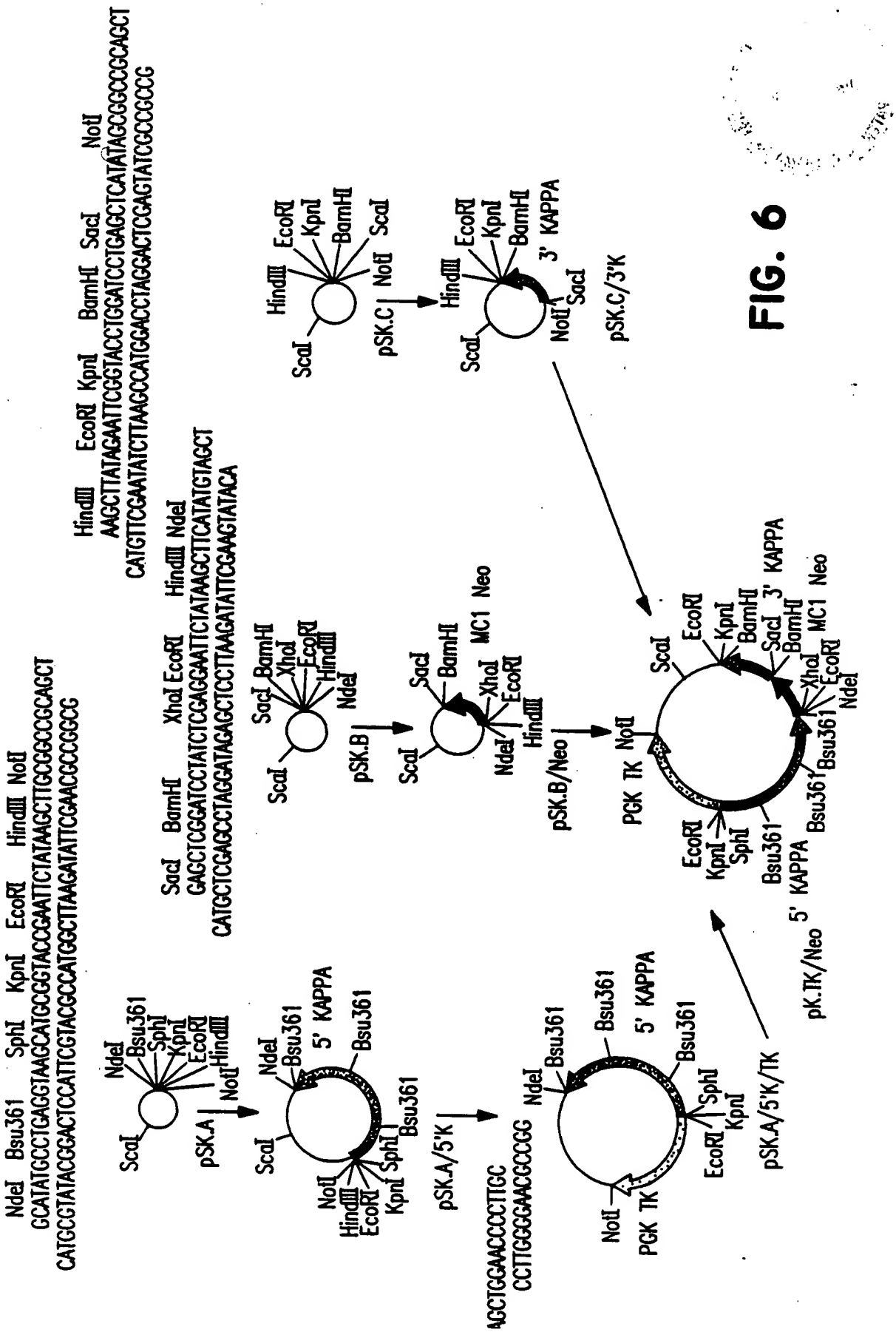

NdeI Bsu361 SphI KpnI EcoRI HindIII NotI
GCATATGCCTGAGGTAAGCATGCGGTACCGAATTCTATAAGCTTGCGGCCGCAGCT
CATGCGTATACGGACTCCATTCGTACGCCATGGCTTAAGATATTCGAACGCCGGCG
HindIII EcoRI KpnI BamHI SacI NotI
AAGCTTATAGAATTCGGTACCTGAGCTCATATAGCGGCCGCAGCT
CATGTTCGAATATCTTAAGCCATGGACTCGAGTATCGCCGCCC
SacI BamHI XhoI EcoRI HindIII NdeI
GAGCTCGGATCCTATCTCGAGGAATTCTATAAGCTTCATATGTAGCCT
CATGCCTCGAGCCTAGGATAGAGCTCCTTAAGATATTCGAAGTATACA
pSK.A
pSK.A/5'K
pSK.B
pSK.B/Neo
pSK.C
pSK.C/3'K
pK.TK/Neo
pSK.A/5'K/TK
5' KAPPA
3' KAPPA
PGK TK
MC1 Neo
Bsu361
ScaI
NotI
NdeI
SacI
BamHI
XhoI
EcoRI
HindIII
KpnI
SphI

FIG. 6

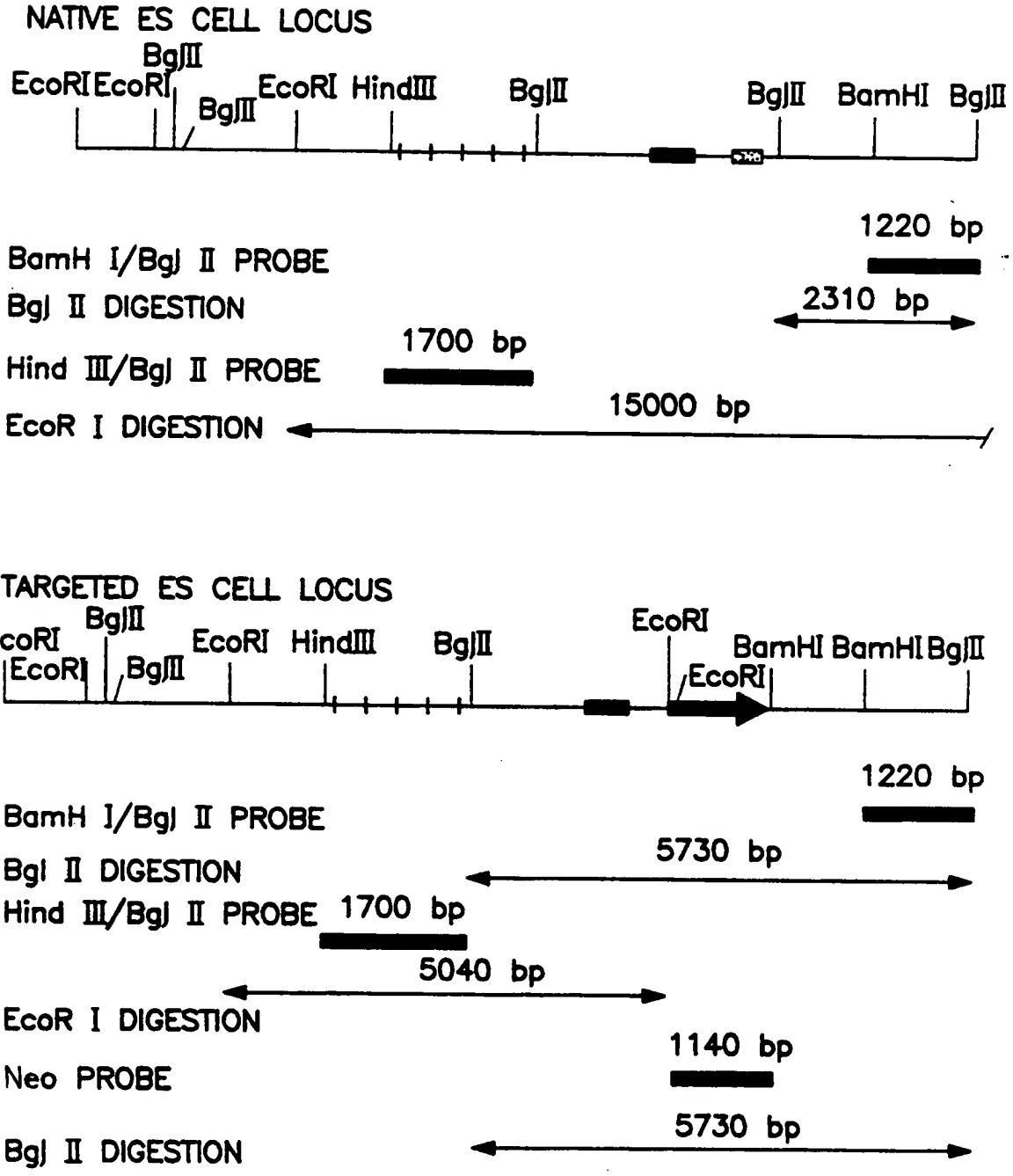

NATIVE ES CELL LOCUS
EcoRI
EcoRI
BglII
BglII
EcoRI
HindIII
BglII
BglII
BamHI
BglII
1220 bp
BamH I/Bgl II PROBE
Bgl II DIGESTION
2310 bp
1700 bp
Hind III/Bgl II PROBE
15000 bp
EcoR I DIGESTION
TARGETED ES CELL LOCUS
EcoRI
EcoRI
BglII
BglII
EcoRI
HindIII
BglII
EcoRI
EcoRI
BamHI
BamHI
BglII
1220 bp
BamH I/Bgl II PROBE
5730 bp
Bgl II DIGESTION
Hind III/Bgl II PROBE
1700 bp
5040 bp
EcoR I DIGESTION
1140 bp
Neo PROBE
5730 bp
Bgl II DIGESTION

FIG. 7

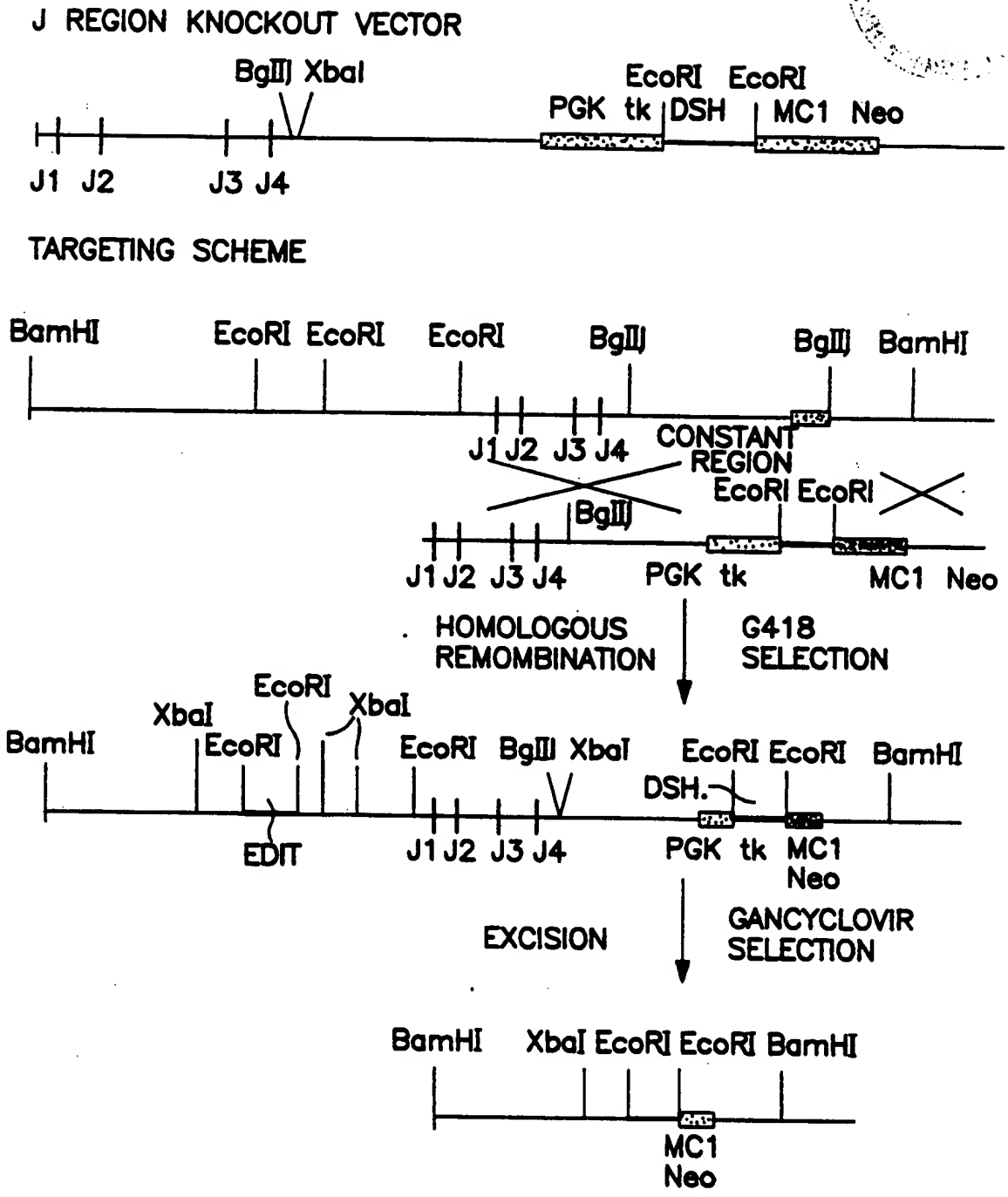

J REGION KNOCKOUT VECTOR
BglII XbaI
EcoRI EcoRI
PGK tk DSH MC1 Neo
J1 J2 J3 J4
TARGETING SCHEME
BamHI
EcoRI EcoRI EcoRI
BglII
BglII BamHI
J1 J2 J3 J4
CONSTANT REGION
EcoRI EcoRI
BglII
J1 J2 J3 J4
PGK tk
MC1 Neo
HOMOLOGOUS REMOMBINATION
G418 SELECTION
EcoRI
XbaI
XbaI
BamHI
EcoRI
EcoRI
BglII XbaI
EcoRI EcoRI
BamHI
DSH.
EDIT
J1 J2 J3 J4
PGK tk MC1 Neo
EXCISION
GANCYCLOVIR SELECTION
BamHI
XbaI EcoRI EcoRI BamHI
MC1 Neo

FIG. 8

SacI BamHI XhoI EcoRI HindIII NdeI
GAGCTCGGATCCTATCTCGAGGAATTCTATAAGCTTCATATGTAGCT
CATCCTCGAGCCTAGGATAGAGCTCCTTAAGATATTCGAAGTATACA

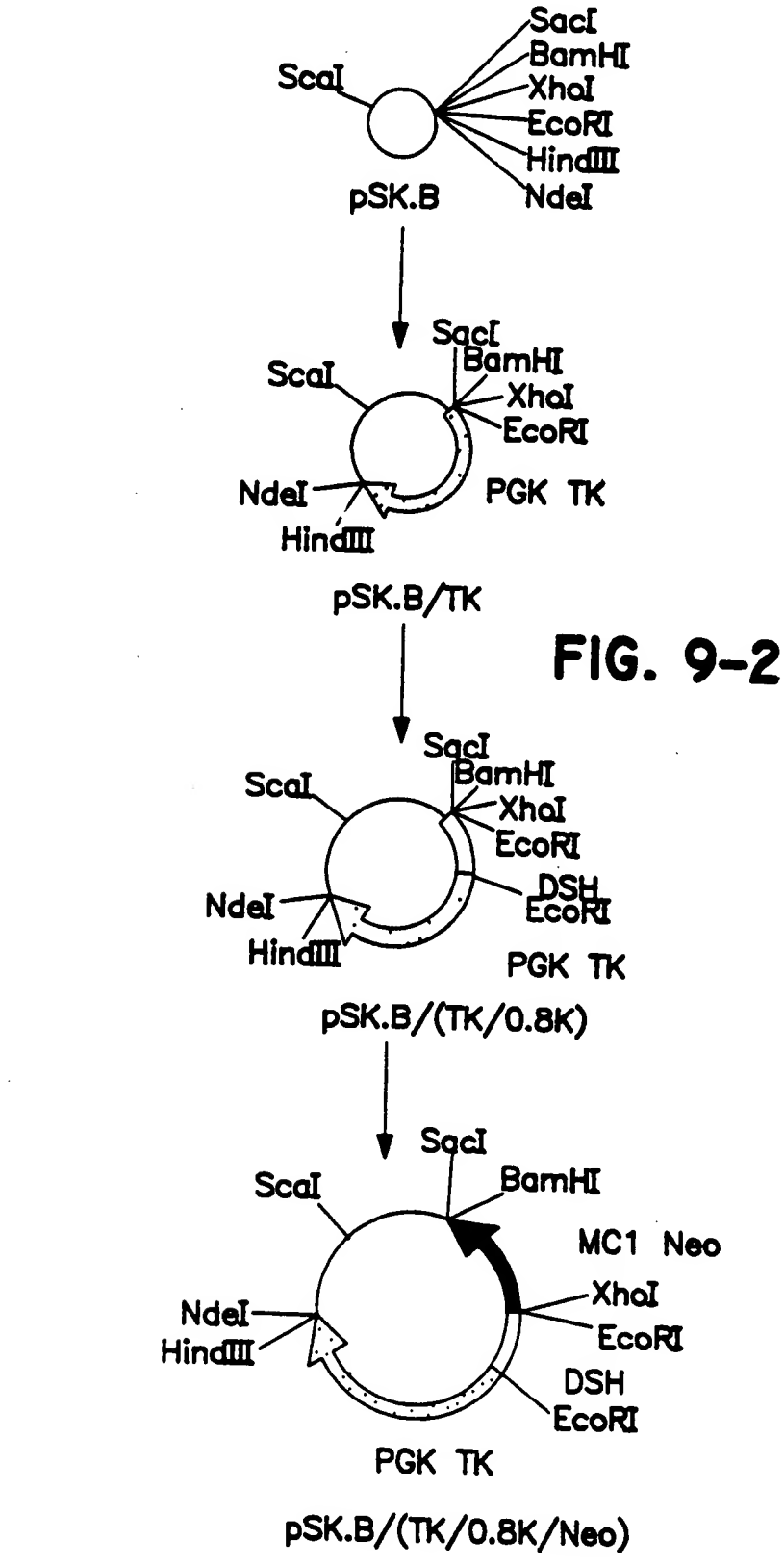



FIG. 9-2

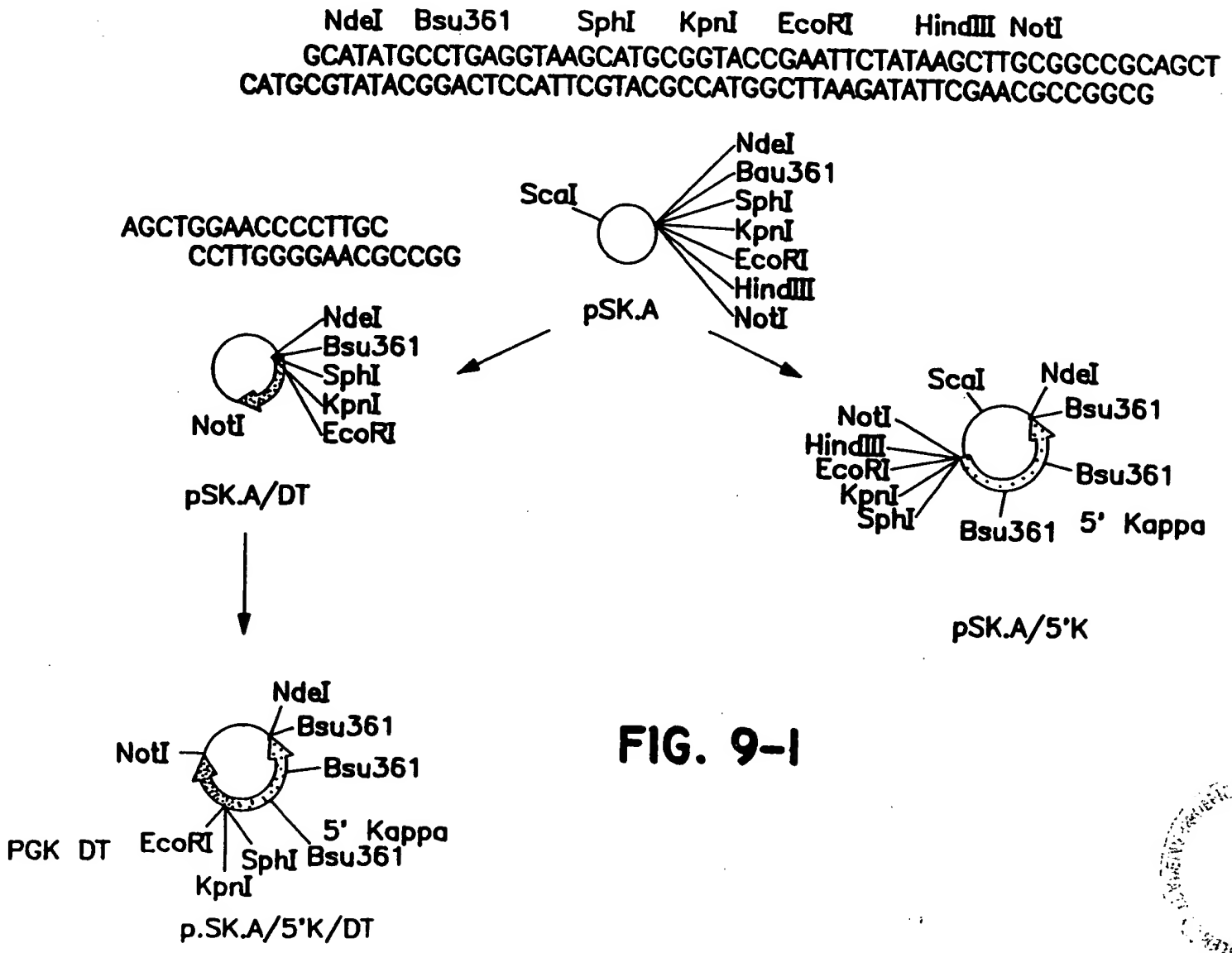
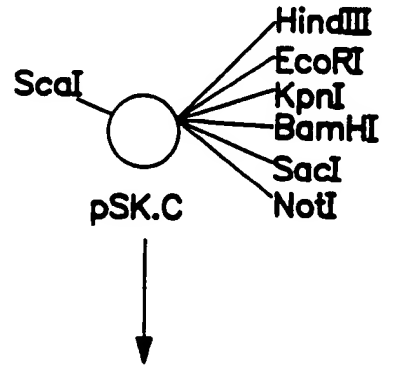

NdeI Bsu361 SphI KpnI EcoRI HindIII NotI
GCATATGCCTGAGGTAAGCATGCGGTACCGAATTCTATAAGCTTGCGGCCGCAGCT
CATGCGTATACGGACTCCATTCGTACGCCATGGCTTAAGATATTCGAACGCCGGCG
AGCTGGAACCCCTTGC
CCTTGGGGAACGCCGG
ScaI
NdeI
Bau361
SphI
KpnI
EcoRI
HindIII
NotI
pSK.A
NdeI
Bsu361
SphI
KpnI
EcoRI
NotI
pSK.A/DT
ScaI
NdeI
Bsu361
NotI
HindIII
EcoRI
KpnI
SphI
Bsu361
Bsu361
5' Kappa
pSK.A/5'K
NdeI
Bsu361
NotI
Bsu361
5' Kappa
PGK DT
EcoRI
SphI
Bsu361
KpnI
p.SK.A/5'K/DT

FIG. 9-1

HindIII EcoRI KpnI BamHI SacI NotI
AAGCTTATAGAATTCGGTACCTGGATCCTGAGCTCATAGCGGCCGCAGCT
CATGTTCGAATATCTTAAGCCATGGACCTAGGACTCGAGTATCGCCGGCG



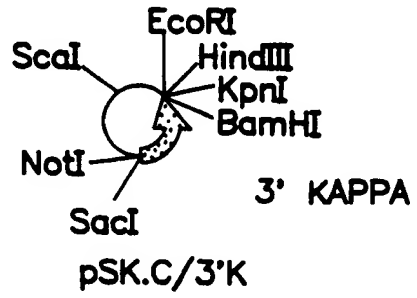
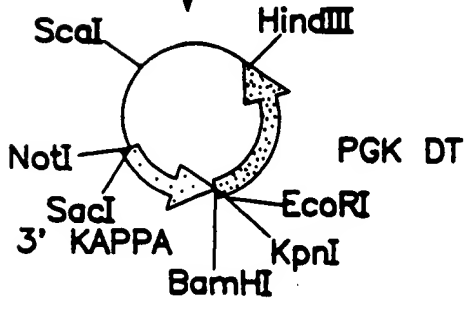




FIG. 9-3



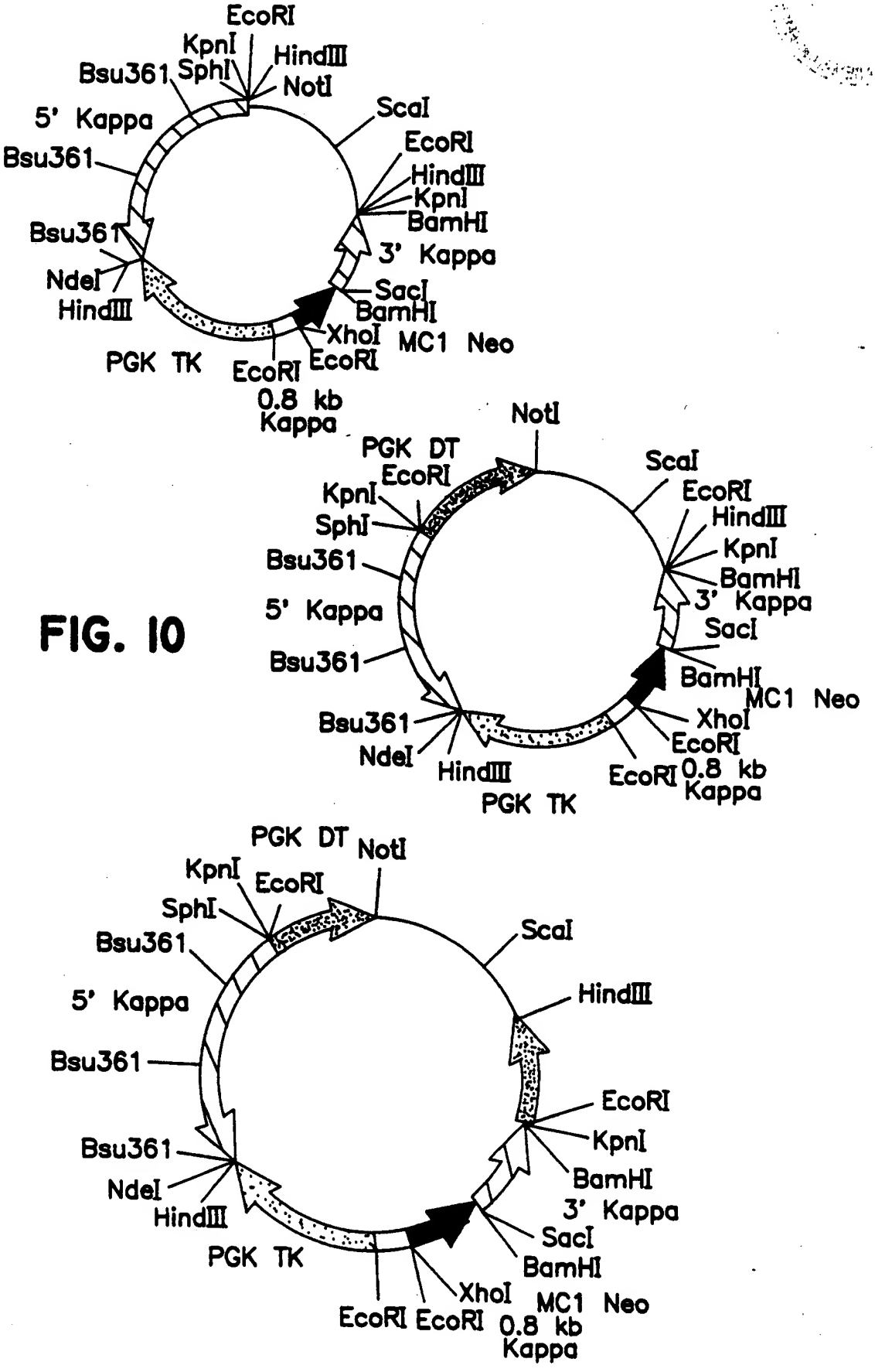

EcoRI
KpnI
HindIII
Bsu361
SphI
NotI
5' Kappa
ScaI
Bsu361
EcoRI
HindIII
KpnI
BamHI
Bsu361
3' Kappa
NdeI
SacI
HindIII
BamHI
XhoI
MC1 Neo
PGK TK
EcoRI
EcoRI
0.8 kb
Kappa
NotI
PGK DT
ScaI
EcoRI
KpnI
EcoRI
HindIII
SphI
KpnI
Bsu361
BamHI
3' Kappa
5' Kappa
SacI
Bsu361
BamHI
MC1 Neo
XhoI
Bsu361
EcoRI
NdeI
HindIII
EcoRI
0.8 kb
Kappa
PGK TK
PGK DT
NotI
KpnI
EcoRI
SphI
ScaI
Bsu361
HindIII
5' Kappa
Bsu361
EcoRI
KpnI
Bsu361
BamHI
3' Kappa
NdeI
SacI
HindIII
BamHI
PGK TK
XhoI
MC1 Neo
EcoRI
EcoRI
0.8 kb
Kappa

FIG. 10

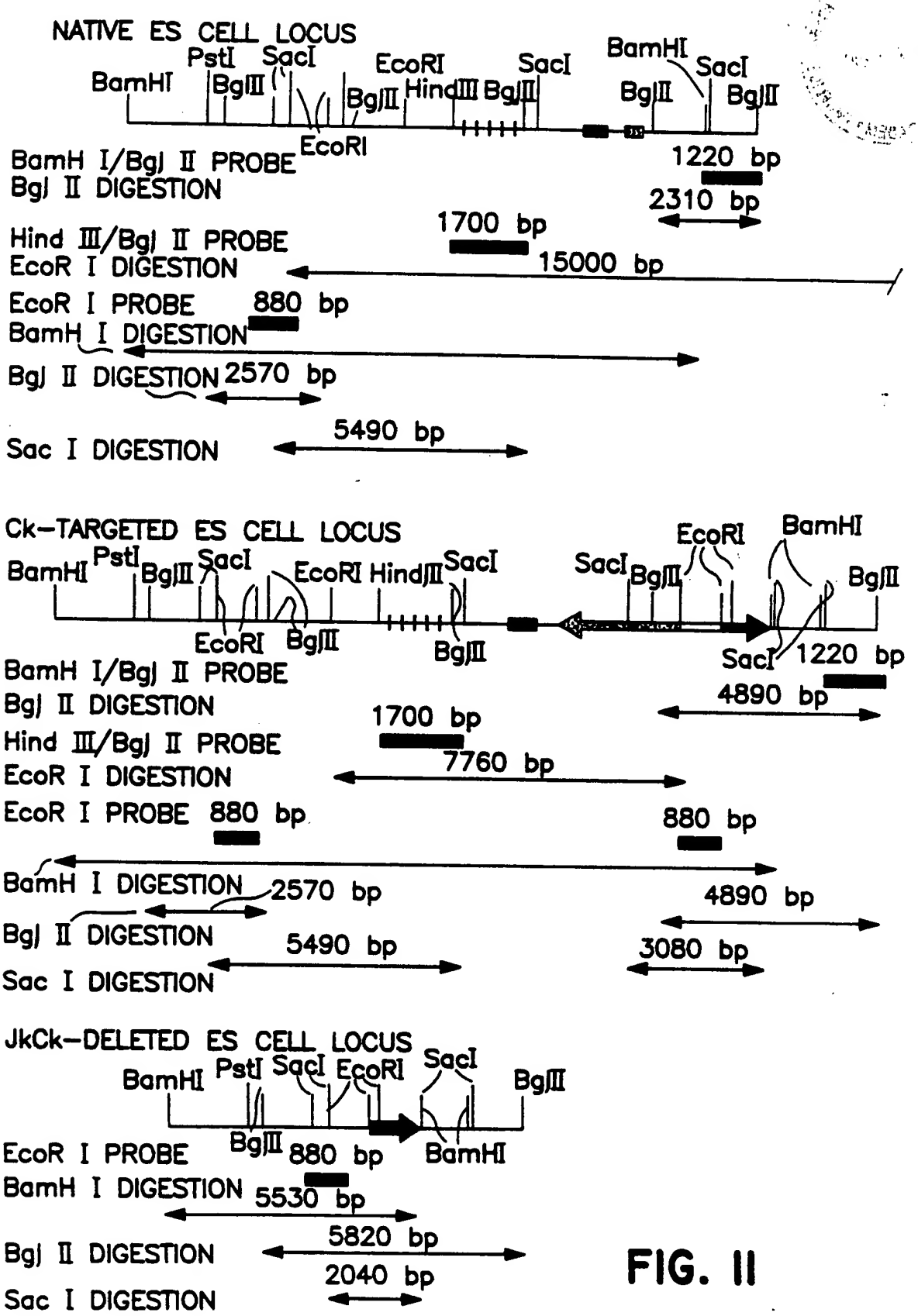

NATIVE ES CELL LOCUS
BamHI
PstI
BglII
SacI
EcoRI
BglII
HindIII
BglII
SacI
BamHI
BglII
SacI
BglII
EcoRI
BamH I/Bgl II PROBE
Bgl II DIGESTION
1220 bp
2310 bp
Hind III/Bgl II PROBE
EcoR I DIGESTION
1700 bp
15000 bp
EcoR I PROBE
BamH I DIGESTION
880 bp
Bgl II DIGESTION
2570 bp
Sac I DIGESTION
5490 bp
Ck-TARGETED ES CELL LOCUS
BamHI
PstI
BglII
SacI
EcoRI
HindIII
SacI
SacI
BglII
EcoRI
BamHI
BglII
EcoRI
BglII
BglII
SacI
BamH I/Bgl II PROBE
Bgl II DIGESTION
1220 bp
4890 bp
Hind III/Bgl II PROBE
EcoR I DIGESTION
1700 bp
7760 bp
EcoR I PROBE
880 bp
880 bp
BamH I DIGESTION
2570 bp
4890 bp
Bgl II DIGESTION
5490 bp
3080 bp
Sac I DIGESTION
JkCk-DELETED ES CELL LOCUS
BamHI
PstI
SacI
EcoRI
SacI
BglII
BglII
EcoR I PROBE
880 bp
BamHI
BamH I DIGESTION
5530 bp
Bgl II DIGESTION
5820 bp
2040 bp
Sac I DIGESTION

FIG. 11

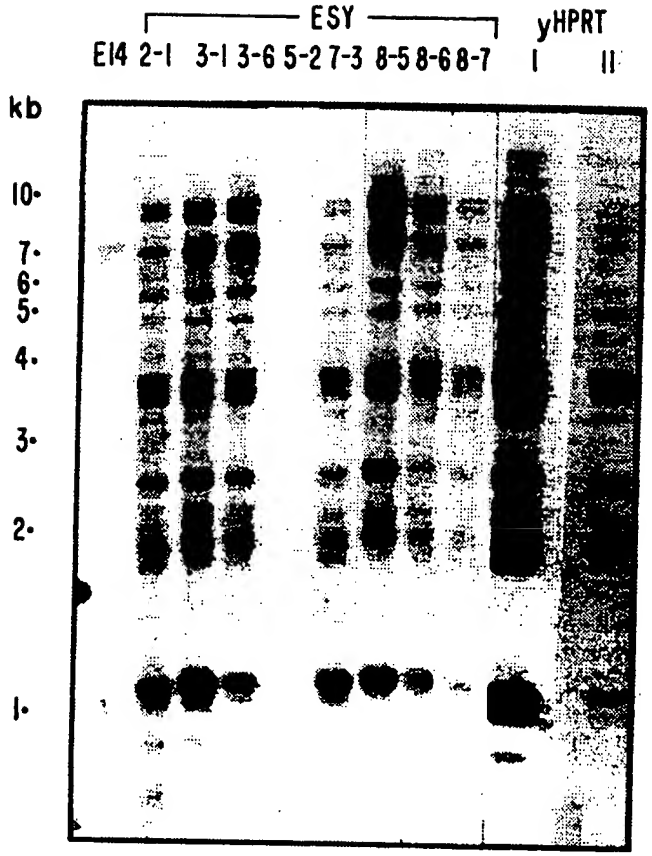



FIG. 12A

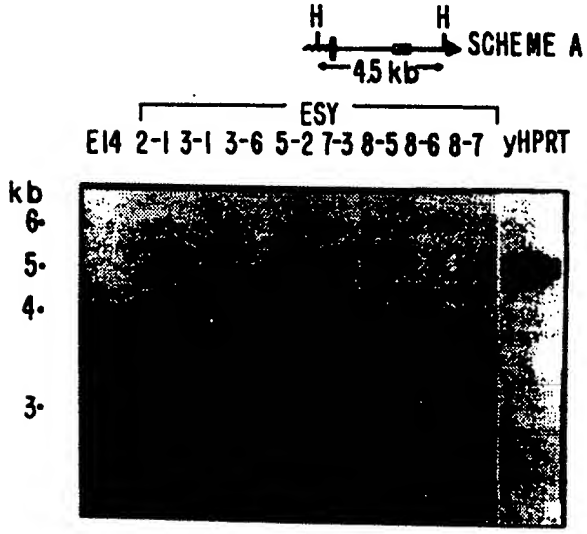



FIG. 12B

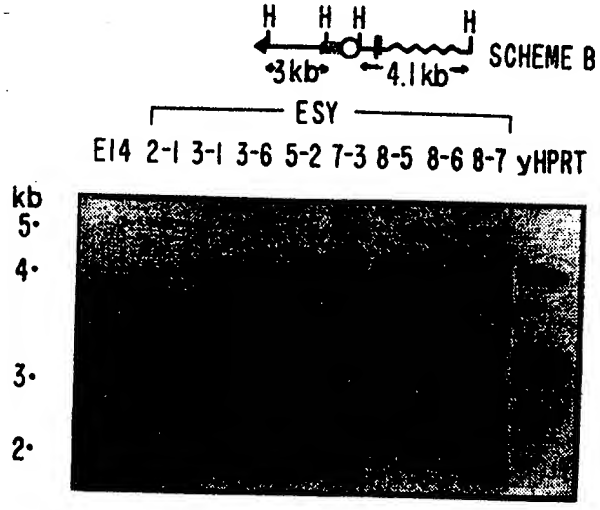



FIG. 12C

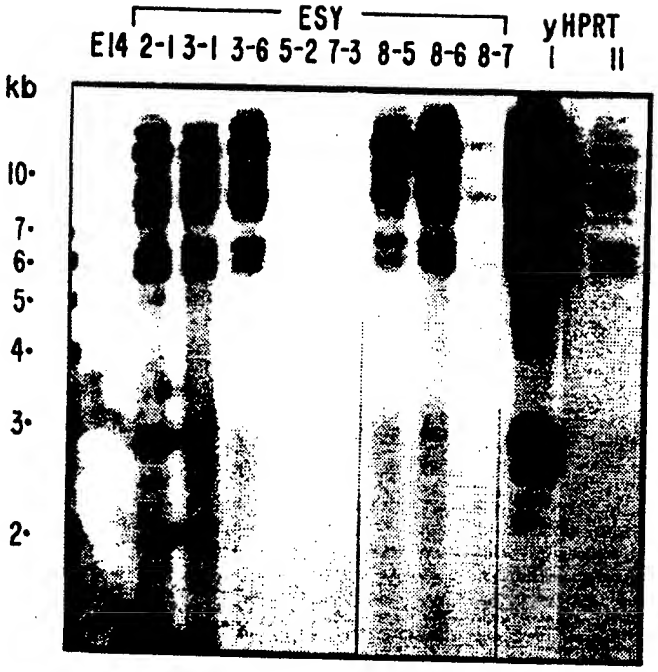



FIG. 12D

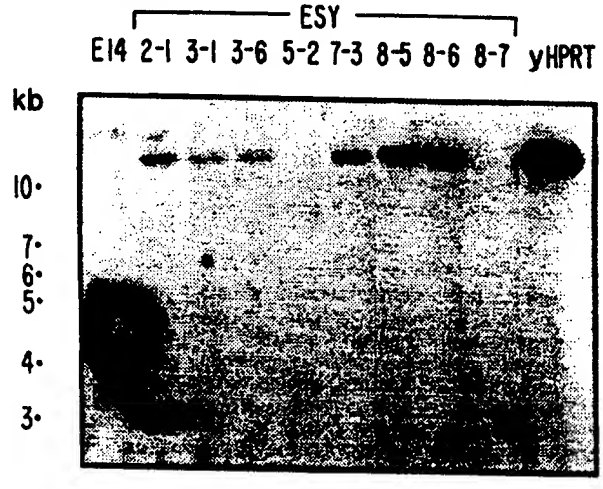



FIG. 12E

FIG. 13A

FIG. 13B

FIG. 13C

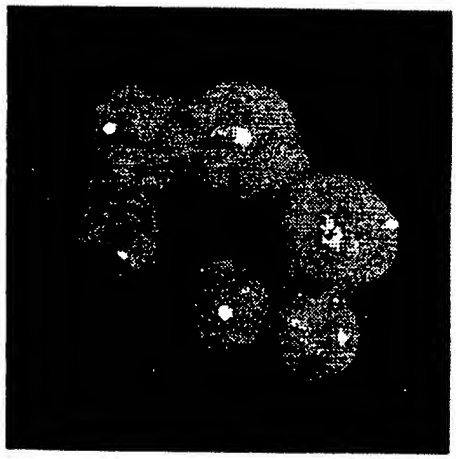


FIG. 13D

FIG. 14A

FIG. 14B

FIG. 14C

FIG. 14D

FIG. 14E

FIG. 14F

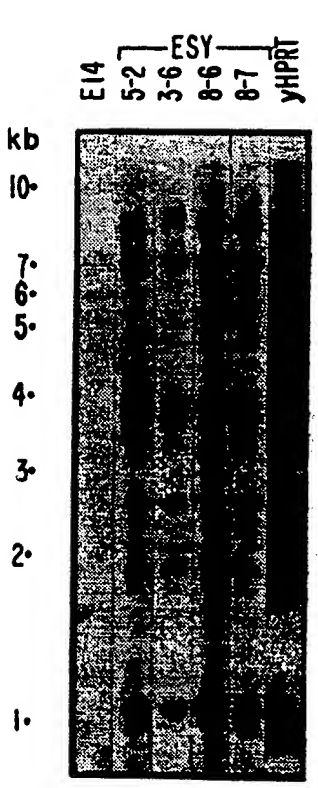



FIG. 14G

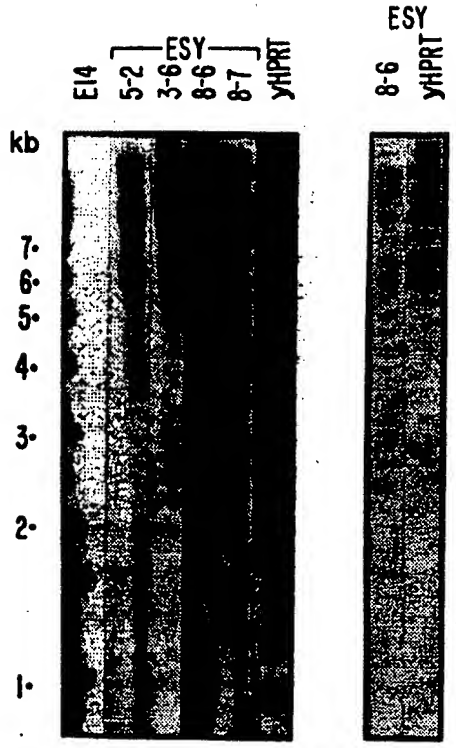



FIG. 14H FIG. 14I

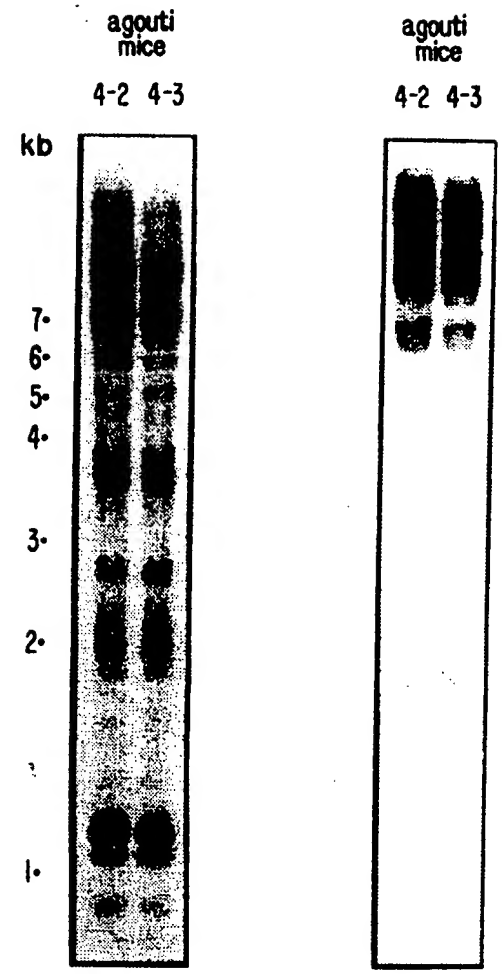



FIG. 14J




FIG. 14K

FIG. 15A

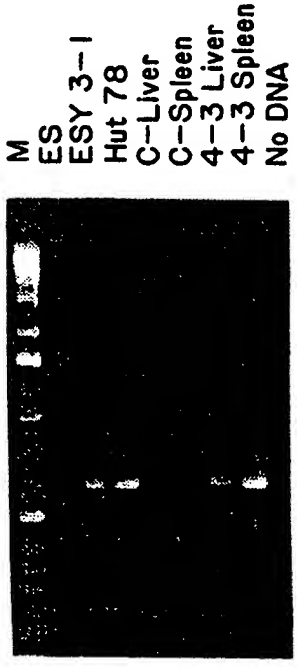
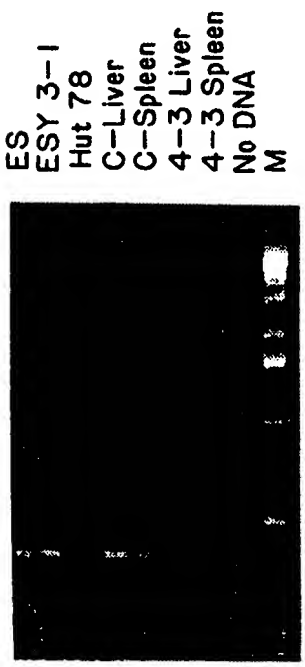



FIG. 15B



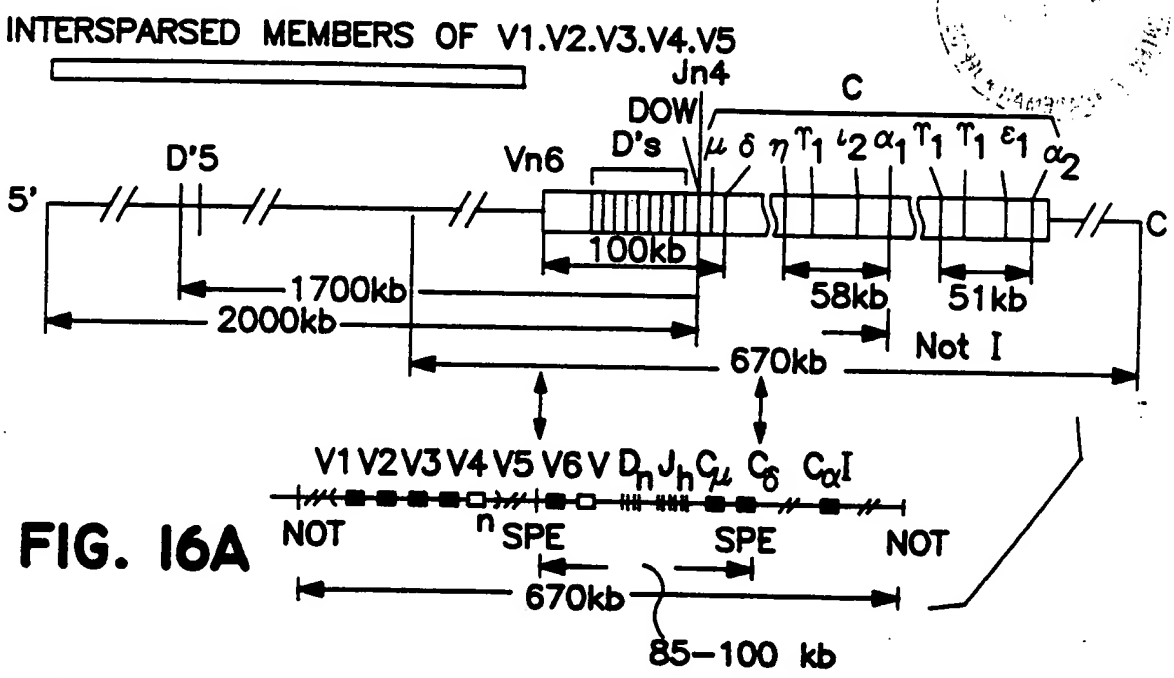



FIG. 16A

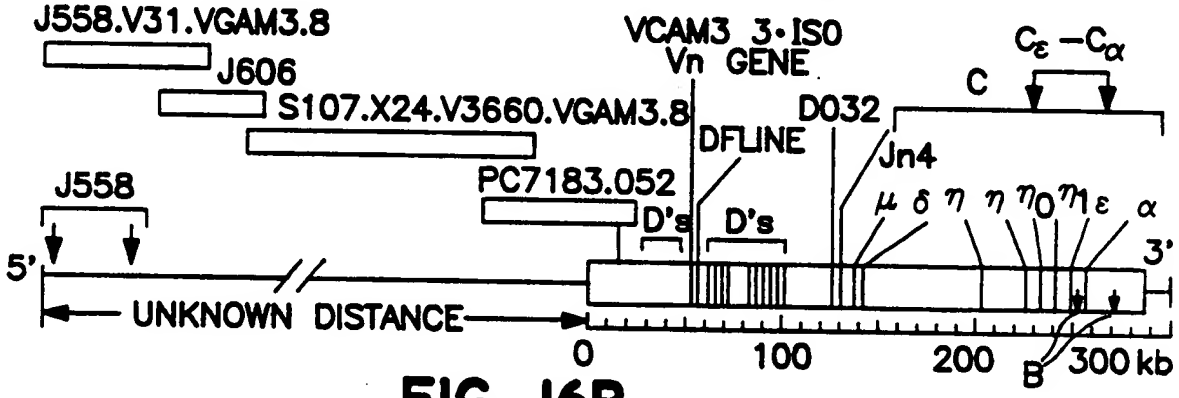



FIG. 16B

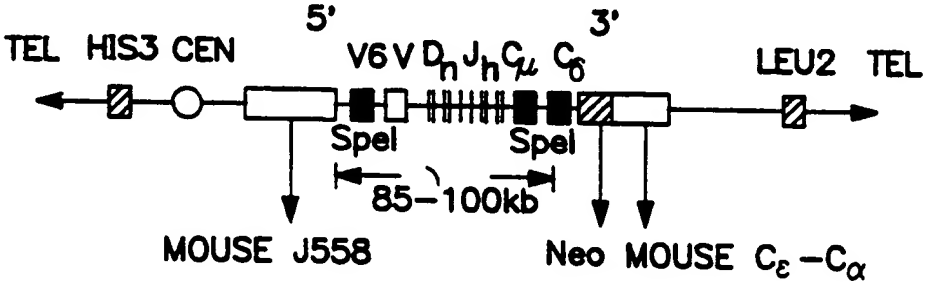



FIG. 16C

SUBSTITUTE

Mouse Breeding Scheme

Cross IA.

heterozygous inactive Murine IgH
X
heterozygous inactive Murine IgK

$\frac{MIgH\ (inactive)}{MIgH}$ $\frac{MIgK}{MIgK}$
X
$\frac{MIgH}{MIgH}$ $\frac{MIgK\ (inactive)}{MIgK}$
↓

F1 (cross I A)

$\frac{MIgH\ (inactive)}{MIgH}$ $\frac{MIgK\ (inactive)}{MIgK}$

Cross IB.

heterozygous Human IgH
X
heterozygous Human IgK

$\frac{MIgH}{MIgH}$ $\frac{MIgK}{MIgK}$ HIgH
X
$\frac{MIgH}{MIgH}$ $\frac{MIgK}{MIgK}$ HIgK
↓

F1 (cross I B)

$\frac{MIgH}{MIgH}$ $\frac{MIgK}{MIgK}$ HIgH HIgK

Cross II. F1 (cross I A) x F1 (cross I B)
↓

F2 Quadruple Heterozygotes

$\frac{MIgH\ (inactive)}{MIgH}$ $\frac{MIgK\ (inactive)}{MIgK}$ HIgH HIgK

Cross III. Intercross F2 mice
↓

F3 DOUBLE Homozygotes

$\frac{MIgH\ (inactive)}{MIgH\ (inactive)}$ $\frac{MIgK\ (inactive)}{MIgK\ (inactive)}$ HIgH HIgK

FIG. 17

## MAMMALIAN HOST GENOTYPES

FIG. 18A

| | Hetero- or Hemi-zygous Mice | Intercross Product Mice * |
|---|---|---|
| I. | $\frac{\Delta mIgL}{mIgL}\ \frac{mIgH}{mIgH}$ | $\frac{\Delta mIgL}{\Delta mIgL}\ \frac{mIgH}{mIgH}$ |
| II. | $\frac{mIgL}{mIgL}\ \frac{\Delta mIgH}{mIgH}$ | $\frac{mIgL}{mIgL}\ \frac{\Delta mIgH}{\Delta mIgH}$ |
| III. | $\frac{mIgL}{mIgL}\ \frac{mIgH}{mIgH}\ \underline{hIgH}$ | $\frac{mIgL}{mIgL}\ \frac{mIgH}{mIgH}\ \frac{hIgH}{hIgH}$ |
| IV. | $\frac{mIgL}{mIgL}\ \frac{mIgH}{mIgH}\ \underline{hIgL}$ | $\frac{mIgL}{mIgL}\ \frac{mIgH}{mIgH}\ \frac{hIgL}{hIgL}$ |
| V. | Animal I X Animal II | |
| | $\frac{\Delta mIgL}{mIgL}\ \frac{mIgH}{\Delta mIgH}$ | $\frac{\Delta mIgL}{\Delta mIgL}\ \frac{\Delta mIgH}{\Delta mIgH}$ |
| VI. | Animal III X Animal V | |
| | $\frac{mIgL}{\Delta mIgL}\ \frac{mIgH}{\Delta mIgH}\ \underline{hIgH}$ | $\frac{\Delta mIgL}{\Delta mIgL}\ \frac{\Delta mIgH}{\Delta mIgH}\ \frac{hIgH}{hIgH}$ and $\frac{\Delta mIgL}{\Delta mIgL}\ \frac{\Delta mIgH}{\Delta mIgH}\ \underline{hIgH}$ |
| VII. | Animal IV X Animal V | |
| | $\frac{mIgL}{\Delta mIgL}\ \frac{mIgH}{\Delta mIgH}\ \underline{hIgL}$ | $\frac{\Delta mIgL}{\Delta mIgL}\ \frac{\Delta mIgH}{\Delta mIgH}\ \frac{hIgL}{hIgL}$ and $\frac{\Delta mIgL}{\Delta mIgL}\ \frac{\Delta mIgH}{\Delta mIgH}\ \underline{hIgL}$ |
| VIII. | Animal VI X Animal VII | |
| | $\frac{\Delta mIgL}{\Delta mIgL}\ \frac{\Delta mIgH}{\Delta mIgH}\ \underline{hIgL}\ \underline{hIgH}$ | $\frac{\Delta mIgL}{\Delta mIgL}\ \frac{\Delta mIgH}{\Delta mIgH}\ \frac{hIgL}{hIgL}\ \frac{hIgH}{hIgH}$ |
| | $\frac{mIgL}{\Delta mIgL}\ \frac{mIgH}{\Delta mIgH}\ \underline{hIgL}\ \underline{hIgH}$ | $\frac{\Delta mIgL}{\Delta mIgL}\ \frac{\Delta mIgH}{\Delta mIgH}\ \frac{hIgL}{hIgL}\ \frac{hIgH}{hIgH}$ and $\frac{\Delta mIgL}{\Delta mIgL}\ \frac{\Delta mIgH}{\Delta mIgH}\ \underline{hIgL}\ \underline{hIgH}$ |

IX. Animal III X Animal IV

$\frac{mIgL}{mIgL}\ \frac{mIgH}{mIgH}\ \frac{hIgL}{}\ \frac{hIgH}{}$ $\frac{mIgL}{mIgL}\ \frac{mIgH}{mIgH}\ \frac{hIgL}{hIgL}\ \frac{hIgH}{hIgH}$

X. Animal II X Animal IX

$\frac{mIgL}{mIgL}\ \frac{\Delta mIgH}{mIgH}\ \frac{hIgL}{}\ \frac{hIgH}{}$ $\frac{mIgL}{mIgL}\ \frac{\Delta mIgH}{\Delta mIgH}\ \frac{hIgL}{hIgL}\ \frac{hIgH}{hIgH}$ and $\frac{mIgL}{mIgL}\ \frac{\Delta mIgH}{\Delta mIgH}\ \frac{hIgL}{}\ \frac{hIgH}{}$

XI. Animal I X Animal IX

$\frac{\Delta mIgL}{mIgL}\ \frac{mIgH}{mIgH}\ \frac{hIgL}{}\ \frac{hIgH}{}$ $\frac{\Delta mIgL}{\Delta mIgL}\ \frac{mIgH}{mIgH}\ \frac{hIgL}{hIgL}\ \frac{hIgH}{hIgH}$ and $\frac{\Delta mIgL}{\Delta mIgL}\ \frac{mIgH}{mIgH}\ \frac{hIgL}{}\ \frac{hIgH}{}$

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*Not all possible genotypes from intercrosses are shown.

Δ = functionally inactive locus
m = mouse endogenous gene
h = human transgene
IgH = immunoglobulin heavy chain
IgL = immunoglobulin light chain

FIG. 18B